Propagation methods for black locust (*Robinia pseudoacacia* L.) improvement in Hungary

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Abstract: Black locust (*Robinia pseudoacacia* L.) is one of the most important stand-forming tree species in Hungary and its importance is increasing in many countries. Black locust plants are commonly produced by two methods, by seed and by root cuttings. Tissue culture propagation can be considered as a relatively new method. Growing trees from seed is a relatively simple method for reliably producing seedlings on a large scale under a variety of circumstances. Mechanization of the method is easy and the production cost is relatively low. Propagation from root cuttings and tissue culture are valuable for reproduction of superior individuals or varieties. By applying these methods, superior traits of the selected trees can be preserved in the clones. Recent experiments demonstrated that micropropagated trees could be successfully transplanted into soil, hardened and grown in the field.

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Introduction

The black locust was the first forest tree species to be imported from North America to Europe at the beginning of the 17th century. Its rapid spread all over the world may be attributed to its adaptability to a wide range of conditions, favorable breeding properties, excellent coppicing, high survival percentage of plantlets, fast growing and high yield, successful using for wood production, honey production and ornamental purposes, as well as good mollification and, not in the least, to the fact that it is damaged only by few diseases and insects. In Hungary the black locust is one of the most important stand-forming tree species, covering approximately 20% of the forested lands (340 thousand hectares) and providing about 18% of the annual timber output of the country (Rédei 1998).

Black locust timber can be used by industry (mining, construction and furniture) or by agriculture sector (post and pole wood). Moreover, the black locust stands are the main basis for Hungarian apiculture and honey production. The black locust is one of the most suitable tree species for establishing energy and environmental plantations as well. The most important black locust growing regions in Hungary are located in south and south-west Transdanubia (hill-ridges of Vas-Zala county, hill-ridges Somogy county), the Danube-Tisza Interfluve (central Hungary) and north-east Hungary (Nyírség region) (Fig. 1). In the future, there are two bigger regions, where the fast spread of black locust can be expected. In Europe the Mediterranean countries (Italy, Greece, Spain and Turkey), while in Asia China and Korea may be the most prominent black locust growers.

Fig. 1 The main growing regions of black locust (*Robinia pseudoacacia L.*) stands in Hungary

According to yield table completed by Rédei (1998), the black locust stands of Yield Class I–II have a rotation of 5–40 years and an annual increment of total volume of 12–14 $m^3 \cdot hm^{-2} \cdot a^{-1}$. The stands of Yield Class III–IV have a rotation of 30 years and an annual increment of 8–9 $m^3 \cdot hm^{-2} \cdot a^{-1}$. The poorest stands (Yield Class V–VI) have a rotation of 20–25 years and an annual increment of 4–6 $m^3 \cdot hm^{-2} \cdot a^{-1}$. In first generation coppice stands, growing

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stock, increment and health are similar to those in high forests.

Geographic distribution and variability

Within the genus *Robinia* the species *R. pseudoacacia* L. black or common locust, and some of its varieties (shipmast locust-var. *pyramidalis)*, and spineless locust -*Ceinwemia* are promising for forest tree breeding. Other varieties are rewarding with respect to breeding for honey production: early flowering (*var. praecox*), late flowering (var. *galiana*) and continuously flowering (var. *semperflorens* Car.), in addition two late flowering and abundantly nectarine species, *R. luxurians* (rich locust) and *R. viscosa* (sticky locust); and hybrids of the latter two species with black locust.

The species is indigenous to the eastern and central part of USA between latitudes 43° and 35° N. In its natural range tree types of black locust are distinguished by shape:

- Pinnata (feathered) type: the stem is straight. It occurs along the northern edge of the species' area at the elevation of about 800 m with *Picea rubra* and *Acer saccarinum.*
- Palmata (palm-like) type: the main part of the stem is crooked and not clearly visible in the crown. Its natural area is in the medium elevations of the Appalachians.
- Spreading type: it seems to be unsuitable for selection. It occurs at the low elevations of the Appalachians, and in the southern part of the natural area.

Brief summery of selective breeding and improvement of black locust in Hungary

In Hungary the main goals of the first black locust selective breeding programme were to improve new clones and cultivars to provide good quality and volume of industrial wood. During this programme superior tree groups were identified in high quality seed stands. Graft material was taken from the plus trees and planted in clone tests at Gödöllő (near Budapest). Mono- and multiclonal varieties were developed and a seed orchard established from the selections. The Hungarian Forest Research Institute is a coordinator of this project.

In Hungary the ranges of sites optimal for black locust growing is rather limited. Black locust growing is exercised often on sub-optimal sites. Moreover, opportunities for black locust growing are highly influenced by climatic conditions and extremes (temperature and precipitation, water supply and unfavourable soil conditions). On the lowlands that are seem to be the most valuable regions for black locust growing the annual precipitation is not more than 500–550 mm, and most of which is outside the growing season. In the summer period the drought is thus a frequently phenomenon, coupled with very high atmospheric temperatures (30–35°C). Relative air humidity in July is usually between 20% and 50%. Due to the filling up of basin-like lowlands in Hungary, site conditions are a mosaic, which changes even over small distances, causing widely differentiated growth potential for black locust plantations. For this reason, there are no large, contiguous lands of homogenous site quality for black locust, and their growth and productivity may be very different inside a large field.

Therefore, the main aim of the new selection work is to find and improve black locust clones (such as 'Kéleshalom 56A 2', 'Császártöltés 61A 3', etc.) which perform good shape, provide good-quality wood material for industrial purposes and can be adapted to the changed ecological conditions as well (Rédei *et al.* 2000) (Table 1).

Methods

Propagation from seed

Black locust is traditionally propagated by sowing seed. Generative way of propagation may gain importance in perspectives when seed orchards and forest stands established with improved cultivars are able to produce seed in significant quantities.

There are two state-approved seed production stands-regions meeting the requirements for black locust seeds (One in Danube–Tiszaa Interfluve Region and the other in Nyírség). The use of seed from other sites is not permitted. Seeds are collected by sieving the top 20 cm of soil beneath the selected seed-production stands. As the seeds of black locust can remain dormant in the soil for several years, the age of seed lots collected in this way is very variable. This is why viability and germination are so variable. Therefore, before sowing, an accurate seed tests is necessary. Seed production for sowing and scarification is carried out by the agency responsible for collecting the seed. It is advisable to treat the seeds against fungi, and this can be done in a small concrete mixer.

Raising black locust plants profitably is possible on loam or sandy loam with a hygroscopicity of between 0.8%–3.5%, a pH between 5.5 and 7.5, and $CaCO_3$ content not exceeding 5%. It is desirable that the phosphorus content be least 15–20 mg/100 g and the easily soluble potassium content be 10–15 mg/100 g at minimum. In the cases if these values are lower, phosphorus and potassium should be added in the form of chemical fertilizers.

The success of raising seedlings is influenced considerably by soil preparation and cultivation. After spreading chemical fertilizer and/ or manure in autumn, it is desirable to plough the area in 35–40 cm depth before winter sets in. Preventive chemical weed control is advisable, and if infection caused by nematodes is traceable, the soil should be sterilized.

Seedbeds for sowing need to be prepared with care. A table-smooth surface is the primary condition for sowing at the same depth. Germination of black locust's hard-shelled seeds is facilitated by pouring boiling water on them, by scarification, or by treating them with sulfuric acid before sowing.

Seeds prepared by one of the above-mentioned methods

should be placed manually or by a machine in a groove 5–8 cm wide and about 3 cm deep. Sow 40–50 seeds of 100 % germination ability per running meter and soil cover is thinner or thicker, dependable emergence can not be ensured.

Table 1. Data of black locust superior trees selected for clonal propagation by tissue culture

Location (sub-compartment)	№ of tree	Age /a	Height /m	dbh_1 /cm	dbh_2 /cm	h_{stem} /m	l_{crown} /m	d_{c1} /m	d_{c2} /m
Pusztavacs 182 A	1	32	26,0	26,2	26,5	18,2	7,8	4,9	4,6
	2	32	27,5	28,2	29,5	17,5	10,0	5,2	4,0
Pusztavacs 201 E	1	33	24,8	28,0	27,8	16,2	8,6	6,5	6,0
	2	33	24,1	28,5	29,2	16,6	7,5	3,9	5,3
	3	33	22,2	21,3	21,4	14,8	7,4	3,9	3,4
Pusztavacs 210 A	1	29	22,0	24,6	23,7	14,5	7,5	4,9	4,8
	2	29	21,5	19,8	19,5	13,8	7,7	4,4	4,2
	3	29	23,0	24,4	23,5	17,0	6,0	4,2	4,5
Pusztavacs 233 A	1	32	21,3	22,0	22,3	14,6	6,7	3,6	4,7
	2	32	23,4	22,5	26,4	15,8	7,6	4,9	3,3
	3	32	22,8	23,2	25,3	15,3	7,5	4,0	4,2
	4	32	23,6	22,5	21,0	15,4	8,2	3,8	3,4
Mikebuda 15 A	1	29	21,0	20,8	21,0	14,0	7,0	5,2	4,6
	2	29	21,5	20,5	19,6	13,5	8,0	4,2	4,3
	3	29	20,0	23,1	23,4	11,5	8,5	4,9	4,0
Mikebuda 17 D	1	27	22,5	25,0	25,7	12,5	10,0	4,0	4,5
	2	27	22,5	23,5	23,7	13,5	9,0	5,2	5,6
	3	27	21,5	21,4	22,2	12,5	9,0	3,4	5,6
	4	27	22,0	23,7	25,3	14,0	8,0	4,8	5,5
Mikebuda 27 G	1	29	23,2	24,5	24,5	14,5	8,7	4,8	4,6
	2	29	23,8	22,3	23,5	14,5	9,3	4,1	5,1
Kéleshalom 56 A	1	14	16,0	16,8	16,0	12,0	4,0	2,8	2,3
	2	14	17,5	17,4	17,2	12,0	5,5	3,5	3,7
	3	14	17,7	16,6	16,5	12,3	5,4	3,0	3,6
	4	14	17,6	17,5	18,3	12,3	5,3	3,9	3,2
Császártöltés 61 A	1	50	26,0	25,4	26,0	18,5	7,5	2,5	2,9
	2	50	26,7	27,5	28,2	19,5	7,2	2,9	2,6
	3	50	26,2	29,0	27,5	17,0	9,2	2,5	4,1
	4	50	26,0	28,8	27,5	16,3	9,7	4,0	3,6

Where: h_{stem} = stem-length to be free of branches; l_{crown} = crown-length; d_{c1}, $_{c2}$ = crown size.

Inter-row spacing of seeds should be chosen in conformity with the working width of the cultivating and weeding machine. According to the recent results, the distance between rows should be at least 35–40 cm. If the spacing is narrower, we should reckon with a decrease in growth. Also, if planted in narrower rows there will be a number of undersized seedlings with poor root systems, making them unsuitable for planting out.

Propagation from root cuttings

The primary requirement for reproducing black locust clones and cultivars was to develop reliable vegetative methods. A working group in the Hungarian Forest Research Institute developed a method for a large-scale production of black locust plants *from root cuttings*, based on the vital sprouting ability of the roots of this species (Keresztesi 1988). For this propagation method, root pieces cut into 8–10 cm, or chopped to 3–5 cm in length, are used. These root pieces should be obtained from the producer of the variety or from a nursery where elite propagation material is produced. The longer root pieces are placed vertically into slits prepared beforehand. The top cut surface of the cuttings should be somewhat below ground level. When plants are silted after planting with a quantity of water corresponding to 10 mm precipitation or more, care is taken so the upper end of the cuttings remain under the soil surface, otherwise they might dry up. At the same time, the cuttings must not be covered with a soil layer greater than 1 cm. If the shoots have to come up from a greater depth the roots themselves or the shoot emergence might be damaged, succumb to fungal infection, and die. In the case of root sowing, root pieces should be sown like oak acorns in a furrow, which is 10 cm wide and 4 cm deep. Twenty-five to 30 root pieces are sown per running meter. The thickness

of soil cover should be uniform and not exceed 4 cm. The thin, short root pieces are planted at a depth of about 4–5 cm. Great attention is given to preventing drying out of the soil at that depth for any length of time. Root pieces for further propagation can be collected from the plants we raised the first year. For this purpose, they should be lifted with as large a root system as possible. It is sufficient to have plants with 3 to 4 root stubs, each 5-cm long, and with rootlets.

In the case of *manual inserting of the root-cuttings* into ground, according to experiments, plant spacing in the rows should be 5–7 cm. With this spacing, 10–15 plants can be raised per running meter. All other roots can be used for further propagation. In general, seedlings are lifted in spring and that is the time when roots for propagation are collected. Normally this takes some 5 to 6 weeks, but if storage of cuttings before planting or sowing is unavoidable, they should be kept permanently moist. When propagating plants from *root pieces*, the appearance of plantlets can be expected 20–25 days after cutting. The average height of the one-year old plants is 1.2–1.5 m.

Plant tissue culture method

Almost sixty new cultivars or selected clones were propagated during the last few years in the Micropropagation Laboratory of Research Institute for Fruit growing and Ornamentals, Budapest in collaboration with the Hungarian Forest Research Institute. *Plant tissue culture methods* provide us with new means to speed up vegetative propagation of recently selected varieties and give us opportunity to establish healthy stock plantations. Brown (1980) was the first to report a successful *in vitro* method for mass production of black locust. Enescu and Jucan (1985) started experiments in Romania in view of similar results. Balla and Vértesy in 1985 had the first success in the sterile production of four Hungarian state-approved black locust cultivars. Balla *et.al* (1998) published the improvement of the acclimation results of micropropagated black locust using symbiotic microorganisms.

Shoot cultures could be most conveniently initiated from actively growing juvenile shoots – e.g. from vegetative stool beds, but most of actively growing shoot tips of the varieties propagated were collected from adult trees, standing in a collection of clones and varieties of the Hungarian Forest Research Institute. May and June, perhaps September is the most suitable time for the culture initiation. The shoot growth is most active in this period of the year. In springtime the disinfections does not mean difficult problems, growing open ground, without any plant protection. Shoot tips of 10–15 mm length, having apical- and lateral buds, without any leaves survive the repeated disinfections in 1% $HgCl_2$ solution containing a few drops of wetting agent (Tween 80) and are not damaged if properly rinsed with sterilized, bidestillated water.

Rooting of black locust clones is usually not successful on the media used for rooting of fruit trees, 1/2 MS containing 1–3 $\mu L \cdot L^{-1}$ of IBA, but added 5 $\mu L \cdot L^{-1}$ of IAA instead of IBA, good results could be achieved. The rate of root formation is 60% to 90 % depending on the cultivars. Acclimation of the black locust clones is performed under greenhouse conditions. The rooted plants are transplanted into Pindstup I (by Pindstrup Mosebrug A/S, Denmark) substrate. The ex-vitro plants need a relatively dry substrate and a high relative humidity to avoid losses by microbial diseases. The glasshouse cabinet used for acclimation purposes should be shaded during summer months. Temperatures above 30 °C and below 15 °C equally may be disastrous. Black locust plantlets root thoroughly into the substrate and start to grow vigorously. The survival rate during the acclimatization is about 70%–80% and can be enhanced with microbial inoculation

The disinfected shoot tips are directly put on the proliferation medium, where about 70% of the cultures are found uncontaminated. Shoot proliferation actually started 4–5 weeks after initiation of the culture, when the shoots are subcultured to the same fresh medium. The lateral buds begin to grow and develop into shootlets. 2–5 new shoots appear monthly at the basis of the older ones.

Micropropagation can be done theoretically all year round, nevertheless it is not advisable to initiate shoot cultures from dormant trees, to acclimatize plants in short-day conditions and keep acclimatized plantlets in the glasshouse for wintering. The multiplication ratios are different depending on the growth vigour of varieties. Plants may be cultivated further in plastic containers or even in soil under field-conditions if provided with an adequate shading and watering system. Plantlets, which are acclimatized till middle of May in the greenhouse and a month transplanted into plastic containers of 3 L outside, under shading and watering conditions, produce 1,5 m big plants that may be planted out in November–December or the following spring. Micropropageted plants of four promising selected clones were planted in a clone trial near Kecskemét (Central-Hungary) in spring 2000. The main ecological conditions of the study area are as follows:

- Humidity is less than 50%
- Hydrology: free draining; genetic soil type: humid sand soil
- The annual precipitation is less than 550 mm (between 1 of April and 26 of September 2000 was: 188 mm)

Special attention was devoted to prevention of root deformation. The seedlings were cut back at about ground level after planting. At the age 1,5 seedlings attained a height of 160–340 cm (plot average). Height field survival (84%–88 %) was achieved (Table 2.). According to the variance analysis of height there was no significant differences between the clones at P=5% level (F_{value}=1.42; $F_{0.05}$=4.07). Clone material regenerated from the same tree showed uniformity in the stem form. Greater variability occurred in height growth of individual trees planted in the field. It seems that nonuniformity in rooting and the development

and number of roots can strongly affect the growth of micro propagated seedlings.

Table 2. Some parameters of micropropagated clone-seedlings at age of 1.5 at Kecskemét

Clone name	№ of plot	Height /m	Height /m (plots average)	Seedlings survival (piece)	Seedlings survival (%)	Survival % (plots average)
	1	3.4		13	81	
'Kéleshalom 56A 2'	9	2.3	2.7	15	94	85
	18	2.6		15	81	
	2	2.9		14	88	
'Kéleshalom 56A 3'	12	2.8	2.5	14	88	84
	15	1.6		12	75	
	3	2.2		13	81	
'Mikebuda 17D 4'	13	1.9	2.0	14	88	88
	16	1.8		15	94	
	5	1.9		14	88	
'Császártöltés 61A 3'	8	2.6	2.0	13	81	88
	14	1.7		15	94	

Conclusions

To conclude, three plant production methods are recommended herein. Application of the seed-sowing method is advisable when the seeds originate from superior black locust stands or seed orchards. The method is simple, can readily be mechanized, its labour consumption is low and, as a consequence, seedlings can be produced at a relatively low cost.

Experiments showed that black locust is a tree species with a great organogenic potential. Use of alternate vegetative plant production methods, propagating plants by cutting or sowing root species and by tissue culture, are advisable for producing clones (varieties). By these methods, the excellent trees of the selected trees can fully be found in the clones. Propagation technology by root cutting need to be developed further because it is labour intensive and the mechanization of the individual processes is only partial. Its application, in spite of that, is recommended for meeting the qualitative requirements of propagation material.

Experiments demonstrated that micropropagated trees could be successfully transplanted into soil, hardened and grown in the field. Micropropagated seedlings exhibited normal growth and appearance with high survival rate at age of 1.

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